

should have spots of corresponding R_f values (0.55 to 0.60).

Subpart H—Tests for Specific Antibiotic Dosage Forms

§ 436.500 Penicillin in oil and wax.

(a) *Potency.* Proceed as directed in § 440.80a(b)(1) of this chapter except paragraph (b)(1)(ix) thereof and, in lieu of the directions in § 440.80a(b)(1)(iv) of this chapter prepare sample as follows: Liquefy the sample by warming, thoroughly mix, and withdraw 1.0 milliliter using a sterile syringe equipped with an 18-gauge needle. Transfer to a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the separatory funnel vigorously to bring about complete mixing of the material with the ether. Shake with a 25-milliliter portion of 1 percent phosphate buffer at pH 6.0. Remove the buffer layer and repeat the extraction with three 25-milliliter quantities of buffer. Combine the extracts and make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. The sample may also be prepared by transferring aseptically 1.0 milliliter of the penicillin in oil and wax to a blending jar containing 100 milliliters of 1 percent phosphate buffer at pH 6.0. Using a high-speed blender, blend this mixture for 1 minute and then make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. If the label represents the potency of the penicillin in oil and wax as 200,000 units per milliliter or less, it is satisfactory if it is 85 percent or more of the potency so represented; if represented as more than 200,000 units per milliliter, it is satisfactory if it is 90 percent or more of the potency so represented.

(b) *Sterility.* Proceed as directed in § 436.20, using the method described in paragraph (e)(2) of that section, except using medium B in lieu of medium A.

(c) *Moisture—(1) Reagents—(i) Karl Fischer reagent.* Preserve the reagent in glass-stoppered bottles and use from an all glass automatic burette, protecting the solution from the moisture in the air.

(ii) *Water-methanol solution.* Use methanol containing approximately 1 mg. of water per milliliter. Store the

solution in a glass bottle attached to an automatic burette and protect from moisture in the air at all times.

(2) *Standardization of Karl Fischer reagent.* Add a known volume of the Karl Fischer reagent to a suitable titrating vessel which has been previously dried at 105° C. and cooled in a desiccator. Introduce a mechanical stirrer and two platinum electrodes which are connected to a suitable electrometric apparatus for measurement of the endpoint. Start the stirrer and titrate with the water-methanol solution until the endpoint is reached. Calculate the milliliters of Karl Fischer reagent equivalent to each milliliter of water-methanol. Add an accurately weighed quantity of water (approximately 50 milligrams) to a dry titrating vessel, add an excess of the Karl Fischer reagent and back titrate with the water-methanol solution as above. Calculate the milligrams of water equivalent to each milliliter of the Karl Fischer reagent. Standardize the Karl Fischer reagent in this manner daily.

$$e = \frac{W}{V_1 - V_2 f}$$

where:

e =milligrams of water equivalent to 1 ml. Karl Fischer reagent.

w =weight of water in milligrams.

v_1 =volume of Karl Fischer reagent used.

v_2 =volume of methanol used.

f =volume ratio of Karl Fischer reagent to water-methanol solution.

(3) *Procedure.* Transfer 1.0 milliliter of the penicillin in oil and wax to a dry titrating vessel, add 10 milliliters of dry chloroform and an excess of the Karl Fischer reagent and back titrate with the water-methanol solution until the endpoint is reached. Transfer 10 milliliters of the dry chloroform used to a dry titrating vessel, add an excess of Karl Fischer reagent, and titrate with the water-methanol as above. Calculate the milliliters of Karl Fischer reagent equivalent to 10 milliliters of chloroform.

$$\text{Percent moisture} = \frac{(V_1 - V_2 f - b) \times e \times 100}{s \times 100}$$

where:

b =milliliters Karl Fischer reagent equivalent to 10 ml. of chloroform.

s=volume of the sample in milliliters.

(d) *Measurement of penicillin particle size.* Vigorously shake the container to obtain an even suspension of the penicillin particles and immediately withdraw therefrom approximately 0.5 milliliter of the drug into a clean, dry, tuberculin syringe using a dry 18-gauge needle. Discard approximately the first 5 drops of the mixture extruded from the needle and then extrude approximately 1 minim of the remaining mixture into a test tube containing 3 to 4 milliliters of light mineral oil. Thoroughly mix the contents of the tube and by means of a bacteriological loop (2 millimeters inside diameter, 22 gauge wire), immediately place one loopful of the suspension on each ruled chamber of a bright line hemocytometer. (It is not necessary to use a cover slip.) Confirm by means of the low power objective of the microscope the even distribution of particles over the ruled areas of both chambers and repeat with another loopful of the suspension if even dispersion is not obtained. Use a magnification of 430 or 440 diameters and a calibrated ocular micrometer to measure the penicillin particles. For the purpose of measurement and calculation, the predominant type of crystals observed shall be considered to represent the type of crystals present and the thickness and density of all particles shall be considered constant. Center a large penicillin particle in the microscopic field; measure the particle and all other particles in the field and repeat this operation on other fields until at least 200 particles are measured. Particles of less than 5 microns in length are disregarded. The grouping of the particles by length, the midpoint, the ratio of the midpoints, and the square of the ratio of the midpoints for each group are tabulated below:

Group	Length in microns	Mid-point	Ratio of mid-points	(Ratio) ²
1	5-14	9.5	1.00	1.00
2	15-29	22.0	2.31	5.34
3	30-49	39.5	4.16	17.31
4	50-69	59.5	6.26	39.19
5	70-99	84.5	8.89	79.03
6	100-149	124.5	13.10	171.61
7	150-199	174.5	18.36	337.09
8	200-249	224.5	23.63	558.38

Group	Length in microns	Mid-point	Ratio of mid-points	(Ratio) ²
9	250-300	275.0	28.95	838.10

Calculate the percent particles in each group from the total number measured. Determine the percent relative weight for each group as follows:

Plate type particles. Relative weight=(ratio)²×% of total particles in group.

$$\% \text{ relative weight} = \frac{\text{Relative weight} \times 100}{\text{Total relative weight}}$$

Rod-shaped particles. In the case of rod-shaped particles measure the width as well as the length.

Relative weight=ratio × average width × % of total particles in group

$$\% \text{ relative weight} = \frac{\text{Relative weight} \times 100}{\text{Total relative weight}}$$

When examined by the method described in this section not less than 50 percent of the total relative weight of the penicillin in the drug consists of penicillin having a particle size of not less than 50 microns in length.

§ 436.503 Procaine penicillin and buffered crystalline penicillin for aqueous injection.

(a) *Total potency (except in single-dose container), sterility, moisture, pyrogens, toxicity, pH.* Proceed as directed in § 440.274b(b) of this chapter.

(b) *Buffered crystalline penicillin content—(1) Preparation of the solution for assay.* Add the indicated amount of distilled water to the contents of a vial of the sample, and shake well. Withdraw one dose of the suspension with a hypodermic syringe and place in a 10-milliliter volumetric flask. Add 20-percent sodium sulfate solution almost to the mark, centrifuge sufficiently to see the meniscus, make to volume with 20-percent sodium sulfate solution, shake well, and centrifuge to obtain a clear or reasonably clear solution. Dilute a 5.0-milliliter aliquot of this clear solution with 1-percent phosphate buffer, pH 6.0, to give a solution for assay of approximately 2,000 units per milliliter.

(2) *Iodometric assay for total penicillin in the solution for assay.* Determine the